

# FROM PARTICLE TRACKING TO MOLECULAR INTERACTIONS

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## ABSTRACT

Live cell imaging often requires single particle tracking to capture the spatio-temporal details of molecular dynamics in situ. Here we present a single-particle tracking algorithm that constructs complete and unbiased trajectories from time-lapse sequences: It first links detected particles frame-by-frame, then closes gaps between initial tracks, accounting for particle appearance, disappearance, merging and splitting. Both steps are compactly formulated as a linear assignment problem, leading to a globally optimal solution. Tracking is supported by on-the-fly classification of particle motion. The tracking algorithm is flexible and versatile. We have applied it to study trans-membrane protein interactions, endocytic pit lifetime distributions and sister kinetochore motion and coupling.

**Index Terms**— single particle tracking, single molecule imaging, gap closing, occlusion

## 1. INTRODUCTION

With the rapid development of bright fluorescent probes and sensitive cameras, live cell imaging has become a standard technique to study the dynamics of sub-cellular objects in situ. The resulting images often consist of punctate objects, for example when imaging sub-resolution macromolecular structures or – in the extreme case – single molecules [1]. In this case, live cell imaging is typically combined with single particle tracking in order to capture the spatio-temporal details of the process of interest [2].

Innumerable commercial and academic solutions to single particle tracking have been proposed [3-6]. Although it is superficially assumed that particle tracking is a solved problem, in reality existing particle tracking algorithms have very limited capability to extract accurate and complete molecular trajectories from image sequences. These algorithms can fail due to several challenges, most notably high particle density, significant heterogeneity in particle motion types, the merging and splitting of particles, and the temporary or permanent appearance and disappearance of particles. These limitations in tracking not only lead to data loss, preventing the optimal use of the great developments in fluorescence microscopy, but also to systematic errors that can result in biased and misleading conclusions regarding the molecular process analyzed.

## 2. METHODS

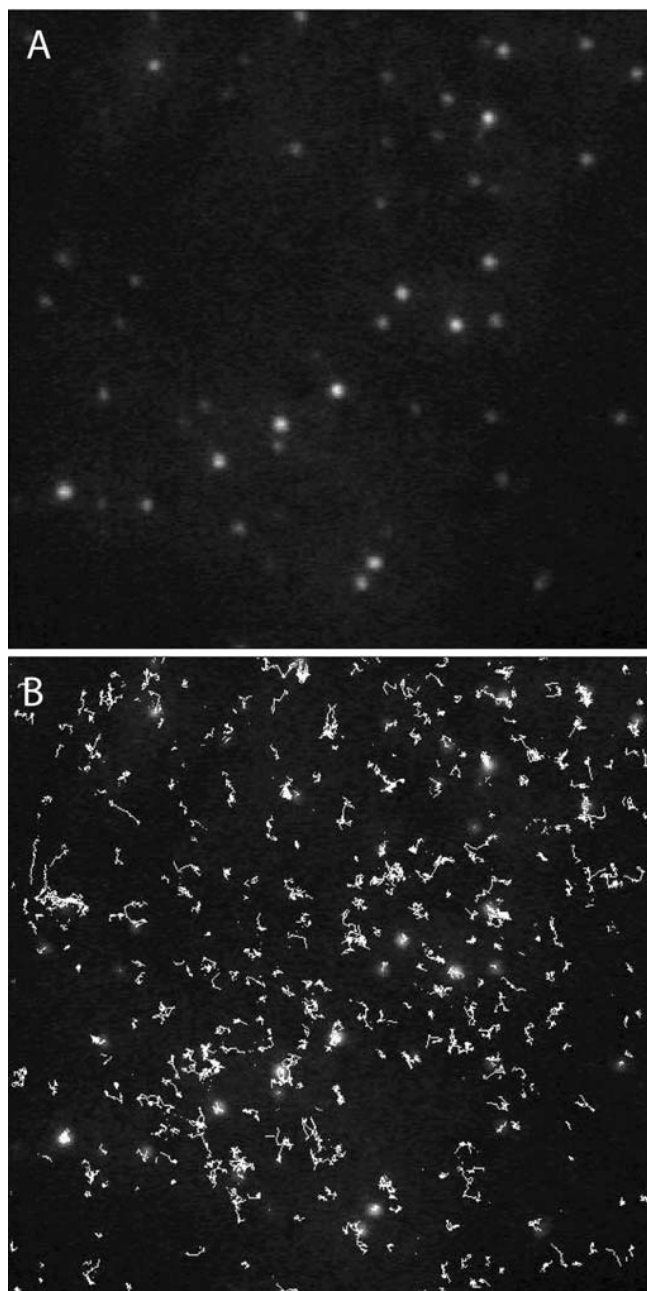
Here we present a tracking algorithm that addresses all of these issues, providing an accurate solution to the single particle tracking problem [7]. To construct complete and unbiased trajectories, our algorithm first links detected particles frame-by-frame and then closes gaps between initial tracks, accounting for particle appearance, disappearance, merging and splitting. Both steps are formulated as linear assignment problems in a bipartite graph [8], leading to a globally optimal solution to both particle assignment in the first step and track assignment in the second step. This general framework is independent of the cost matrix used for assignment; consequently, the cost matrix is flexible and can be tailored to the problem of interest. Furthermore, the algorithm can be applied to problems of various dimensions.

For our applications, the cost for a link between two particles is taken to be proportional to the distance between them, potentially after explicit propagation of a particle's position given the non-Brownian aspect of its motion (e.g. preferential motion along a certain direction). The motion modeling is very flexible: Particles can obey different motion types and can switch between them over the course of a trajectory. The cost for closing a gap, i.e. linking one track's end to another track's start, is also based on distance, taking into account the non-Brownian aspects of the tracks. The cost for merging and splitting events depends not only on distance but also on particle intensities. This ensures that merging and splitting events are not picked up only due to the proximity of particles but that the associated intensity changes are consistent with the image superposition of merging or splitting particles.

## 3. RESULTS AND DISCUSSION

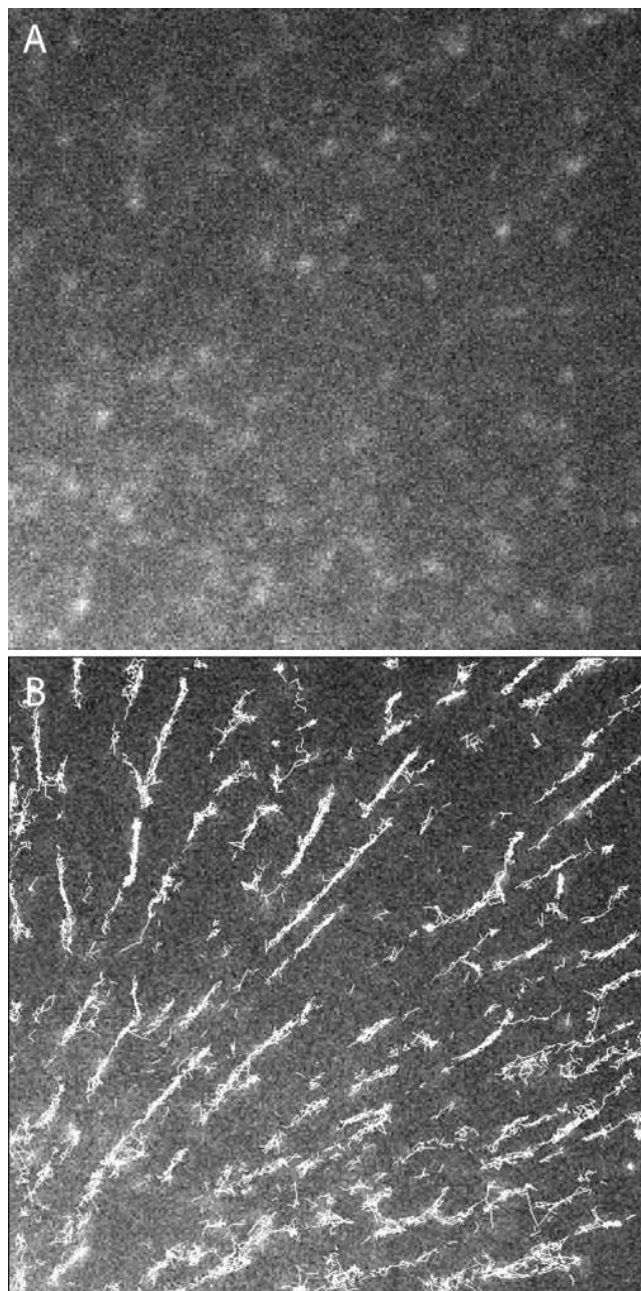
### 3.1. Endocytic pit lifetime and functionality

The goal of this study was to measure the lifetimes of endocytic clathrin-coated pits (CCPs) in the membrane and to correlate their lifetimes with their functionality. We used TIRF microscopy to image EGFP-labeled CCPs in BSC1



**Figure 1.** Endocytic CCPs. (A) A sample image of EGFP-tagged CCPs taken via TIRF microscopy. (B) CCP tracks of the movie shown in (A).

cells (Fig. 1A) that were then detected using the a-troux algorithm [9] and tracked with the appropriate time window to close gaps (Fig. 1B). Accurate tracking that delivered complete tracks with properly closed gaps was critical to this study, since failure to close gaps would chop tracks into shorter segments and systematically bias the lifetime distribution toward smaller values. Detailed analysis of the obtained lifetime distribution revealed that there were three populations of CCPs with different endocytic productivity and characteristic lifetime in the membrane. These data led

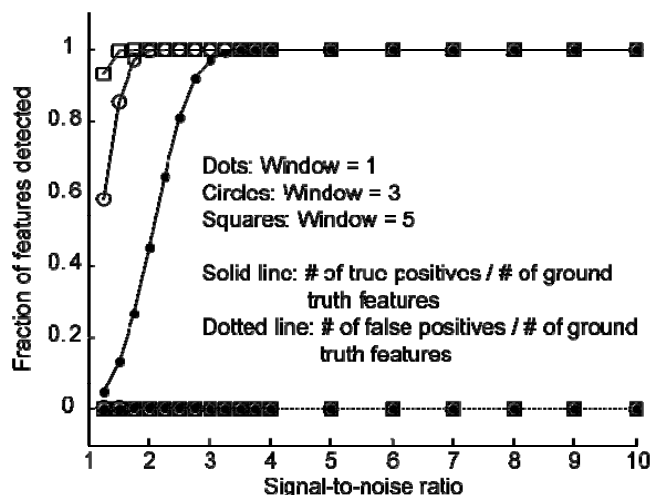


**Figure 2.** The macrophage trans-membrane protein CD36. (A) A sample image of Cy3-labeled CD36 molecules taken via epi-fluorescence microscopy. (B) CD36 tracks of the movie shown in (A).

to the discovery of an endocytic checkpoint that monitors the readiness of a CCP for internalization.

### 3.2. Trans-membrane protein interactions

The goal of this study was to characterize the kinematics of and interactions between the macrophage trans-membrane receptor CD36, the clustering of which is thought to be important for its signaling function. Here it was not only



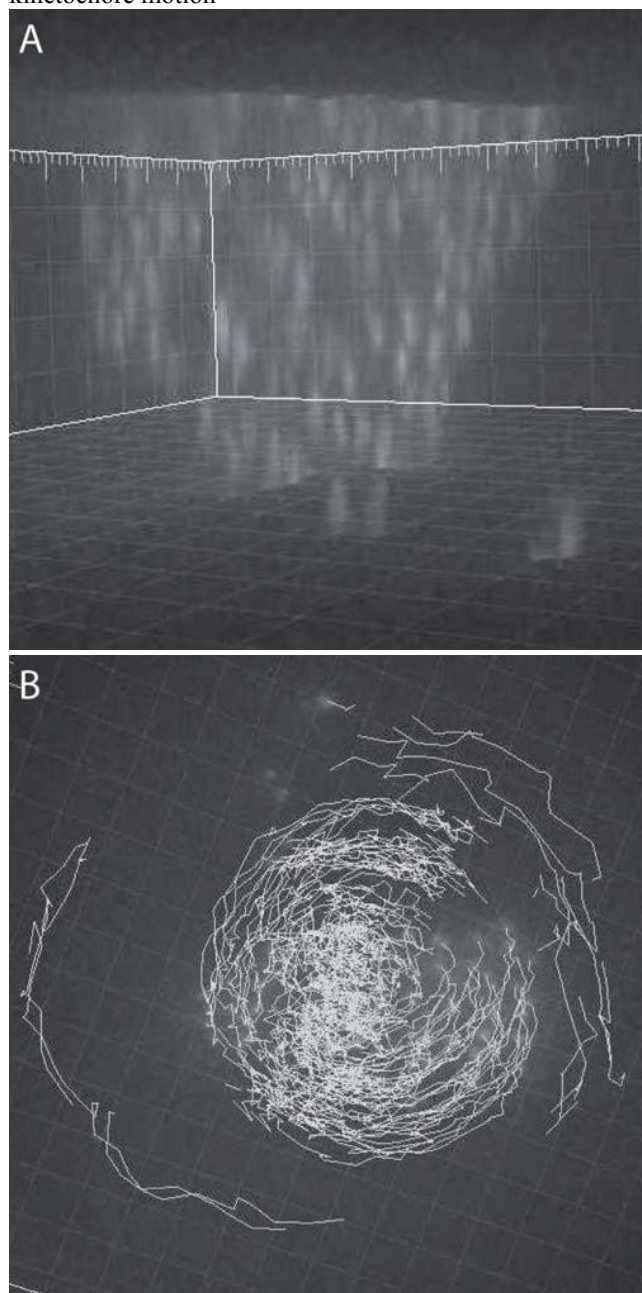
**Figure 3.** Time-averaging enhanced detection efficiency. Features placed on a regular grid against backgrounds of increasing noise were detected and the detection results were compared to the ground truth. Detection with time averaging means that the search for local maxima was done on time-averaged images using a sliding window of a certain value. A window of 1 implies that there was no time averaging. Signal-to-noise ratio was defined as the ratio of signal above background to the standard deviation of background intensity.

critical to get complete tracks, but to also capture merge and split events that indicated interactions between individual CD36 molecules. We obtained single molecule images of Cy3-labeled CD36 in primary macrophages using epi-fluorescence microscopy (Fig. 2A), and then estimated their positions by fitting Gaussian kernels in areas around local maxima [10]. To enhance detection efficiency under the low signal-to-noise ratio conditions of single molecule movies, the search for local maxima prior to Gaussian fitting in individual frames was performed in time-averaged images using a sliding window of 3 (Fig. 3). Since CD36 molecules seemed to move along linear tracks, we tracked them by explicitly including in the cost function information on speed and directionality (Fig. 2B). Interestingly, we found that the CD36 molecules that moved along linear tracks had twice the probability of merging and splitting, i.e. interacting, than the molecules that did not move on linear tracks. These observations define a novel function of cytoskeleton structures in organizing receptor trafficking to enhance the chance for accumulation of signaling-competent clusters.

### 3.3. Sister kinetochore motion and coupling

The goal of this study was to characterize the motion of and coupling between sister-kinetochores during cell division and to elucidate the roles of various kinetochore proteins in their regulation. While imaging in the previous two applications was limited to 2D, in this study it was

necessary to image and track in 3D. We followed kinetochore motion



**Figure 4.** HeLa kinetochores. (A) A sample image of EGFP-labeled kinetochores taken via epi-fluorescence microscopy. (B) Kinetochore tracks of the movie shown in (A).

in HeLa cells by labeling a kinetochore protein with EGFP and imaging via epi-fluorescence microscopy (Fig. 4A). We estimated the 3D kinetochore positions by simple local maxima detection and centroid calculation. Then we tracked kinetochore positions over time using an appropriate time window that resulted in complete kinetochore trajectories (Fig. 4B). These trajectories were used to identify pairs of sister kinetochores and to analyze properties of force

transmission between kinetochore pairs. This study revealed that sister kinetochore breathing and the oscillation of kinetochores about the metaphase plate is regulated by many kinetochore proteins.

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